Colony PCR

- First alternative:
  - Pick one colony with a sterile tip and elute it in 100 µl ddH_2O or medium
  - Store the colony at 4 °C while colony PCR is running
- Second alternative:
  - Pick one colony with a sterile tip and streak cells at a marked position on a new plate
  - Put tip in PCR tube already containing the reaction mixture
- For both alternatives continue as follows:
  - One reaction mix contains:
    - 5 µl 5x buffer
    - 1 µl $MgCl_2$ (25 mM stock)
    - 0.5 µl 10mM dNTPs
    - 0.25 µl primer mix (prefix/suffix primers or sequencing primers)
    - 17.625 µl ddH_2O
    - 0.125 µl GoTaq polymerase (Promega)
    - 0.5 µl template
- PCR program:
- Cell lysis and initial denaturation: 5 min, 95 °C
- 30 cycles of:
  - 10 s, 95 °C
  - 30 s, annealing temperature
  - 1 min / 1 kb of expected product, 72 °C
  - Final elongation: 5 min, 72 °C
- Gel electrophoresis: check the fragment size
- First (as above) alternative:
  - Plate the correct colony
- Second (as above) alternative:
  - Use cells from the right positions to start liquid cultures or streak them on a new plate